



# ELISA based Monitoring and Quantification of Tetracycline Residues in Fresh and Powdered Cow Milk Commercialized in Constantine Region (Northeast Algeria)

L. Boultif<sup>1</sup>, N. Zeghilet<sup>2</sup>, M.C. Abdeldjelil<sup>1</sup>, A. Boudebza<sup>1</sup>, B. Chebira<sup>1</sup>

10.18805/ajdfr.DRF-343

## ABSTRACT

**Background:** This study aimed to identify tetracycline residues in milk consumed in the region of Constantine (northeast Algeria) using ELISA based tetracyclin kit.

**Methods:** A total of 180 samples were analyzed (fresh cow milk and imported powdered milk). To compare ELISA and HPLC detection values, 22 fresh milk ELISA positive samples were confirmed by HPLC analysis.

**Result:** 92.5% of fresh milk samples contained tetracycline residues at values between 5 and 74 µg/L and 33.3% of the samples showed concentrations between 49 and 74 µg/L, that exceed the MRLs recommended by the FDA. No significant differences ( $p > 0.01$ ) were found between the values obtained by the two methods.

**Key words:** Antibiotic residues, ELISA, Fresh milk, Powdered milk, Tetracycline.

## INTRODUCTION

The presence of antibiotic residues in animal products has always been a source of concern for human and animal health, as well as for the dairy industry. Antibiotics are regularly used for therapeutic or prophylactic purposes (Kümmerer, 2001; Shaheen *et al.*, 2016; Sabbya *et al.*, 2019; Valarmathi *et al.*, 2020). Tetracycline is one of the most widely used molecules in livestock breeding, particularly for the treatment of respiratory, genital and foot diseases (Furusawa, 2003; Sarmah *et al.*, 2006; Desalegne, 2011; Van Boeckela *et al.*, 2015).

Since Tetracycline is a molecule excreted in milk (Zahid Hosen *et al.*, 2010), it is very likely to be found as residues. It is also one of the most resistant antibiotics in the field, both in humans and animals (Anurag *et al.*, 2021; Ramasamy *et al.*, 2021; Vatalia *et al.*, 2021).

In Algeria milk is one of the most consumed animal products, for many consumers, it represents the main source of animal protein. Nevertheless, local authorities have not yet rigorously applied international standards or established specifications for residue limits in milk commercialized in the country. The rapid implementation of such guidelines is necessary to protect the consumer's health and limit losses incurred by the dairy processing industries. There are several methods for the qualitative detection of antibiotic residues in milk. These methods are mainly microbiological methods. (Bhusal *et al.*, 2020; Zhang and Wang, 2009; Franekand Diblikove, 2006), other methods are used for residue quantification such as high performance liquid chromatography or mass spectrophotometry (Khachatourians *et al.*, 1998, Simonsen *et al.*, 1998; Khan *et al.*, 2020; Waghamare *et al.*, 2020; Dawadi *et al.*, 2021; Al-Kindi *et al.*, 2023). The wide use of such methods is limited by many factors, such as complex sample preparation and procedures, the necessity of qualified personnel, time-consuming and high costs (da Conceição Luiz

<sup>1</sup>PADESCA Research Laboratory, Institute of Veterinary Sciences, Mentouri Constantine 1 University, Constantine, Algeria.

<sup>2</sup>GSPA Research Laboratory, Institute of Veterinary Sciences, Mentouri Constantine 1 University, Constantine, Algeria.

**Corresponding Author:** L. Boultif, PADESCA Research Laboratory, Institute of Veterinary Sciences, Mentouri Constantine 1 University, Constantine, Algeria. Email: [boultif.latifa@umc.edu.dz](mailto:boultif.latifa@umc.edu.dz)

**How to cite this article:** Boultif, L., Zeghilet, N., Abdeldjelil, M.C., Boudebza, A. and Chebira, B. (2024). ELISA based Monitoring and Quantification of Tetracycline Residues in Fresh and Powdered Cow Milk Commercialized in Constantine Region (Northeast Algeria). Asian Journal of Dairy and Food Research. doi: 10.18805/ajdfr.DRF-343.

**Submitted:** 10-07-2023 **Accepted:** 20-03-2024 **Online:** 03-05-2024

*et al.*, 2018). Nowadays, immunoassays such as ELISA (enzyme-linked immunosorbent assay) as well as microbial test kits are commonly used for milk sample testing (Li *et al.*, 2017; Mukunzi *et al.*, 2017; Shen *et al.*, 2019). ELISA is among the latest adapted methods used to detect antibiotic residues. This method is extremely sensitive, simple and able to screen a large number of samples (Clifford, 1985; Szekacs, 1994; Gardner *et al.*, 1996; Lee *et al.*, 2000). This study aimed to monitor and quantify the residual levels of tetracyclines in milk consumed in the region of Constantine (northeastern Algeria). The ELISA-based analysis concerned locally produced cow milk and imported powder milk.

## MATERIALS AND METHODS

### Milk samples

#### Collection of milk samples

120 samples of fresh cow's milk and 60 samples of powdered milk were collected in the region of Constantine (northeast

Algeria) from March to June 2022. The collection was based on a stratified random sampling method. The population was divided into strata and samples were collected within each region in a homogeneous but random fashion. Fresh cow milk samples were collected aseptically and transported to the laboratory in isothermal bins at 4°C and stored in a freezer at -18°C until analysis. This storage method does not affect the antibiotic concentrations, as reported by several authors (Abhishek Gaurav *et al.*, 2014; Ferraz Spisso *et al.*, 2009). The sampling of powdered milk was from the three most consumed brands in the region. All these brands use imported milk. Samples were collected in sterile airtight plastic bags containing 100 g of powdered milk. For each brand, the samples were taken from different batches that could be produced in different countries.

### Milk sample preparation

Milk samples were defatted and then diluted. Raw milk was centrifuged for 10 min at 3000 rpm in a refrigerated centrifuge. The formed upper cream layer was removed and the resulting skimmed milk was diluted using the dilution buffer (ratio 1:10). To minimize interference, several authors use dilutions of 16 × or 20 × before proceeding to the detection of antibiotics by ELISA (Aga *et al.*, 2003; Dolliver *et al.*, 2008). Milk powder samples were diluted: 10 g of milk powder in 100 mL of preheated (60°C) distilled water. The mix was shaken for a few minutes with a vortex, then subjected to an ultrasonic bath until the complete dissolution of the powder. The reconstituted milk is defatted and then diluted by mixing 50 µL of the milk with 450 µL of the dilution buffer.

### Tetracycline ELISA kit

#### Components of the kit

Two RIDASCREEN® tetracycline kits (R-Biopharm AG, Darmstadt, Germany) were used. Each kit is composed of a microplate of 96-well (12 strips of 8 divisible wells) coated with tetracycline/protein conjugate. The test kit includes all the reagents and standards required for the enzyme immunoassay: Tetracycline concentrated standards (0 ppb (zero standard), 0.5 ppb, 1.5 ppb, 3 ppb, 6 ppb and 18 ppb); Conjugate; Anti-tetracycline antibody; Pro red chromogen; Stop Solution; Dilution buffer and Wash Buffer.

#### Preparation of standards and solutions

The standards are supplied as concentrates, so they must be diluted at a rate of 50 µl of concentrated standard (supplied in the kit) in 450 µl of dilution buffer and mixed well by vortex.

For the preparation of the phosphate buffer, we dissolved the entire contents in one liter of distilled water. This wash buffer can be kept for about 4-6 weeks at 2-8°C.

### Experimental methodology

#### Principle of the test

The test is based on the antigen-antibody reaction. The microtiter wells are coated with tetracycline-protein conjugate. Tetracycline standards or sample solutions and

anti-tetracycline antibodies are added to the pre-coated wells. Competition between free and immobilized tetracycline for the tetracycline antibody binding sites (competitive enzyme immunoassay) occurs. The unbound antibodies are then removed by the washing step and the enzyme named secondary antibody, which is directed against the anti-tetracycline antibody, is added. After removing the unbound enzyme by the washing step, the substrate/chromogen solution is added to the wells and incubated. The bound enzyme conjugate converts the chromogen to a blue product. The addition of the stop reagent leads to a color change from blue to yellow. Colour intensity is measured photometrically at 450 nm. Absorbance is inversely proportional to the concentration of tetracycline in the sample. It is therefore an indirect competitive ELISA method.

#### Analysis protocol

The RIDASCREEN® kit (Biopharm AG, Darmstadt, Germany) was used following the manufacturer's instructions. The analysis protocol involved the following operations: A volume of 50 µL of the solution (standard or sample) was placed in each well then 50 µL of anti-tetracycline antibody solution was added. The whole was gently mixed and incubated for 1 hr at room temperature (20-25°C). After incubation, the liquid from the wells was emptied by vigorously inverting the microplate and draining against absorbent paper. The washing step was performed using 250 µL of PBS-Tween buffer this operation was repeated twice. In every well, 100 µL of conjugate solution was added, mixed gently then incubated for 15 min at 20 - 25°C. Another washing operation similar to the previous one was performed. 100 µL of chromogen solution was added to each well, mixed and incubated for 15 min at 25°C in the dark. Finally, 100 µL/well of stop solution was added. The reading was made at 450 nm within 30 minutes after the addition of the stop solution.

#### Reading and expression of results

The results were calculated on semi-logarithmic paper. The percentages of absorbance were calculated according to the following formula:

% absorbance =

$$\frac{\text{Absorbance of the standard (or sample)}}{\text{Absorbance of the zero standard}} \times 100$$

Then, the percentages were extrapolated on the concentration curve of the standards. The zero value (of the standard) corresponds to 100% absorbance. For the other values, it decreases with increasing concentrations. To obtain the real concentration in µg/kg of tetracycline, contained in a sample, the concentration taken from the calibration curve must be further multiplied by the dilution factor of 10 for cow's milk and 100 for milk powder (recommendations specified on the assay kit).

#### Comparison of some results obtained by HPLC analysis

Twenty-two positive samples detected by ELISA analysis were analyzed a second time by HPLC (high performance

liquid chromatography). The search was directed to oxytetracycline for which the analytical method has been previously validated (Boultif *et al.*, 2014).

### Statistical analysis

Comparison of values obtained by HPLC analysis and those obtained by ELISA were compared by Student's t test. Results were considered statistically significant at  $p < 0.01$ . The calibration curve and the student test were made by SATISCTICA stat soft V10 software.

## RESULTS AND DISCUSSION

### The standard curve

After reading on a microplate, the values obtained correspond to the absorbance at 450 nm. The absorbance is inversely proportional to the amount of tetracycline present in the samples; the more antibiotic is present, the lower the absorbance. Table 1 illustrates the absorbance of standards from 0 to 180  $\mu\text{g/L}$ .

According to these results, the minimum absorbance (0.22%) corresponds to the highest concentration (180  $\mu\text{g/L}$ ). Thus, if concentrations exceed the maximum detection limit of the test (approximately 200  $\mu\text{g/L}$ ), they will not be accurately quantified. The calibration curve is based on the absorbance percentages of the standards, according to their

respective concentrations. The concentrations of the samples are obtained by extrapolating the percentages of absorbance, on the calibration curve; on semi-logarithmic paper expressed in  $\mu\text{g/L}$ . Fig 1 represents the calibration curve of the kit1.

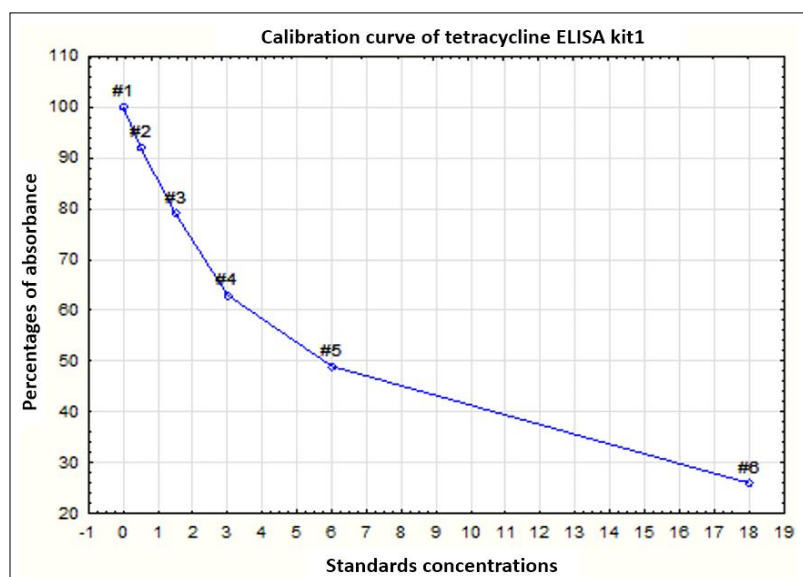
### Tetracycline residue in Fresh milk

Of the 120 fresh milk samples, 7 (7.5%) contained less than 5  $\mu\text{g/L}$ . The remaining samples (92.5%) contained tetracycline residues at values between 5 and 74  $\mu\text{g/L}$ . 33.3% of the samples showed maximum concentrations between 49 and 74  $\mu\text{g/L}$ . Table 2 shows the absorbance limits, as well as the concentrations for each batch.

All the values recorded remain below the maximum residue limit recommended by the Food and Agriculture Organization of the United Nations (FAO), the European Union (EU) and Japan, which have established maximum residue limits (MRLs) of tetracycline in milk at 100  $\mu\text{g/L}$  (Chris *et al.*, 1999; Naoto, 1999). However, the safety level set by the FDA (Food and drug administration) is 30  $\mu\text{g/L}$  (Popadoyannis *et al.*, 2000). A study in 2007 published that in the United States, the maximum residue limit for tetracycline should be 6  $\mu\text{g/L}$  for animal foodstuffs (Moats, 2000; Popadoyannis *et al.*, 2000). 33,3% of our samples largely exceeded this maximum limit.

**Table 1:** Percentage absorbance and concentration of standards.

Standard	N	Percentage of absorbance (%)		Concentration ( $\mu\text{g/L}$ )	
		Minimum	Maximum	Minimum	Maximum
Kit 1	6	26	100	0	180
Kit 2	6	22	100	0	180



**Fig 1:** Calibration curve of ELISA kit 1, representing the concentrations of the standards ( $\mu\text{g/L}$ ) according to the percentages of absorbance.

**Table 2:** Results of fresh milk analysis.

Fresh milk	N	Pourcentage of absorbance (%)		Concentration (µg/L)	
		Minimum	Maximum	Minimum	Maximum
Batch 1	10	71	95	4,5	22
Batch 2	10	78	91	5	15
Batch 3	10	76	99	0	18
Batch 4	10	48	99	0	60
Batch 5	10	39	91	5	74
Batch 6	10	74	90	5,9	24
Batch 7	10	54	99	0	49
Batch 8	10	67	89	6,8	25
Batch 9	10	46	94	4	65
Batch 10	10	65	92	5	9.2
Batch 11	10	47	79	2.5	25
Batch 12	10	54	90	5	15

**Table 3:** Milk powder analysis results.

Milk powder	N	Absorbance percentage (%)		Concentration (µg/L)	
		Min	Max	Min	Max
Batch 1	30	54	97	3	15
Batch 2	15	54	91	5	15
Batch 3	15	54	97	3	15

**Table 4:** Comparison between values obtained by HPLC analysis and those obtained by ELISA.

Sample	HPLC	ELISA
A6	25.42	22
A8	22.25	20
H2	26.74	25
H3	45.98	42
J3	7.74	5.3
J8	64.34	65
J10	14.72	18
D8	22.07	15
E1	42.09	45
E5	29.86	25
E6	52.09	46
F1	35.83	30
F2	20.97	22
F4	44.13	40
F6	35.44	40
F7	50.86	52
F8	62.53	60
F9	15.85	12
G5	17.76	18
G6	76.24	74
G9	31.46	30
G10	17.44	15
Mean±SD	34.62±18,21	32.22±19.18
SEM	3.8834	4.0904

t = 0.4055, p value = 0.6892, p>0.01 not significant.

### Tetracycline residue in milk powder

Of the 60 samples analyzed, only four samples contained less than 5 µg/L (6.6%). The rest (93.4%) had concentrations between 5 and 15 µg/L (Table 3). Nevertheless, these concentrations remain below the maximum limits recommended by various health control bodies.

### Comparison between ELISA and HPLC analysis results

For the 22 ELISA positive samples that were reanalyzed by HPLC, no significant differences were found between the values obtained by the two methods (Table 4).

Table 4 shows an average of 34.62±18.21 µg/l of tetracycline obtained by HPLC compared with 32.22± 19.18 of tetracycline obtained by ELISA. The difference is no significant. So no differences were found between the values obtained by the two methods. Tetracycline can be quantified by ELISA. The method is used worldwide to detect and identify antibiotic residues in milk. The difference in sensitivity between the tests available on the market has a direct impact on the detection of antibiotic residues in milk (Adesiyun *et al.*, 1997). Thus, different situations around the world have been reported.

In Turkey, Ergin Kaya *et al.* (2010) reported that 1.25% of samples tested positive for tetracyclines. Whereas a study of 60 samples by Unusan (2009) revealed that 40 samples contained tetracycline residues but at values below the MRLs. In Korea, out of 478 samples, 5% were found to be positive (Chang-Soo *et al.*, 1996). In Iran, Karamibonari and Movassagh (2011) used the ELISA method to detect Tylosin in pasteurized milk and found that 63% of the samples were

contaminated, but at levels below the MRLs. A study in the Czech Republic indicated that 50.6% of the samples were contaminated but with concentrations below the MRLs (Navratilova *et al.*, 2009). However, in another study in Macedonia, the residue limit for oxytetracycline exceeded 149.1 µg/kg (Elizabeta *et al.*, 2011). In Kuwait, a study on imported and locally produced milk indicated that 29.1% of the samples were contaminated with values above the MRLs (Alomirah *et al.*, 2007). A study in China (Zhang *et al.*, 2014) involving 94 UHT and 26 pasteurized milk samples, respectively showed 0 and 7.7% contaminations at values above MRLs. Finally, Bilandžić *et al.* (2011) worked on 119 samples and the values found were lower than the maximum limit of tetracycline in milk. With a maximum of 49.5 µg/kg and the average concentration found is 35 times lower than the MRLs. From the above-mentioned studies, we can see that the ELISA technique is a qualitative method, which allows the detection of antibiotic residues and thus gives percentages of contamination. This method also allows characterizing the molecule responsible for the contamination and quantifying the antibiotic residues in milk at thresholds lower than the MRLs recommended worldwide. Like all methods, ELISA has limitations. Its dynamic range of detection is quite low and this type of method is quite time-consuming, due to long incubation times (Pietschmann *et al.*, 2020). It also requires experienced and qualified personnel to perform the tests (Molina *et al.*, 2003; Fejzic *et al.*, 2014; Romero *et al.*, 2016). Errors in the test procedure can lead to false results. Such errors include improper storage conditions of the test kit (or reagents), incorrect pipetting sequence or reagent volumes and too long or too short incubation times during the immune and/or substrate reaction. Other errors include extreme temperatures during test performance (below 10°C or above 30°C) and direct exposure to a strong light source. Thus, as with any analytical technique, positive samples that require action must be confirmed by an alternative method.

## CONCLUSION

Enzyme-linked immunosorbent analysis is a valuable method to identify and quantify tetracycline residues in milk. This method is both sensitive and specific for detecting this antibiotic molecule and could replace HPLC assay for this purpose. In fact, it is a less heavy method, requiring less sample preparation and therefore requires less staff. On the other, 33.3% of the analyzed fresh milk samples in our study contained tetracycline residues with values exceeding the recommendations of the FDA (food and drug administration). However, they remain below the MRLs (maximum residue limit) set by other agencies. Our results confirm the widespread use of tetracycline by cattle breeders in Algeria. Such use must be accompanied by rigorous residues monitoring policies.

## Conflict of interest

All authors declared that there is no conflict of interest.

## REFERENCES

- Abhishek, G.J.P.S., Gill, R.S., Bedi, J.S. (2014). ELISA based monitoring and analysis of tetracycline residues I cattle milk in various districts of Punjab. *Veterinary World*. 17: 26-29.
- Adesiyun, A.A. and Balbirsingh, L.A.W (1997). Prevalence of antimicrobial residues in preprocessed and processed cows' milk in trinidad. *J. Food Safety*. 16: 301-310.
- Aga, D.S., Goldfish, R., Kulshrestha, P. (2003). Application of ELISA in determining the fate of tetracyclines in land-applied livestock wastes. *The Analyst*. 128: 658-662.
- Al-Kindi, S., Ahuja, A., Almainani, R., Al Mamari, S.K. and Al-Balushi, M. (2023). Investigation of antibiotics residues in imported and locally produced red meat in Muscat, Sultanate of Oman. *Asian Journal of Dairy and Food Research*. 42(2): 262-266. doi: 10.18805/ajdr.DRF-294.
- Alomirah, H., Al-Mazeedi, H., Al-Zenki, S., Al-Aati, T., Al-Otaibi, J., Al-Batel, M., Sidhu, J. (2007). Pevalence of antimicrobial residues in milk and dairy products in the state of Kuwait. *Journal of Food Quality*. 30: 745-763.
- Anurag, B., Ramasamy, T., Ramesh, S. Sriraam, K.S., Kalaiselvi, L., Deepak, S.J., Kannan, R.G. and Arunaman, C.S. (2021). Screening of milk borne *Staphylococcus aureus* for resistance against beta lactam antibiotics. *Agricultural Science Digest*. 41(1): 113-115. doi: 10.18805/ag.D-5123.
- Bhusal, D.R., Chhetri, B. and Subedi, J.R. (2020). Determination of antibiotics residues in milk samples collected in the different sites of Kathmandu, Nepal. *Asian Journal of Dairy and Food Research*. 39(3): 195-200. doi: 10.18805/ajdr.DR- 186.
- Bilandžić, N., Kolanović, B.S., Varenina, I., Jurković, Z. (2011). Concentrations of veterinary drug residues in milk from individual farms in croatia. *Veterinary Drug Residues in Milk, Mljekarstvo*. 61(3): 260-267.
- Boultif, L., Zeghilet, N., Chebira, B., Agabou, A., Mekroud, A. (2014). Validation of a high performance liquid chromatography (HPLC) method for the determination of oxytetracycline residues in milk. *adv. Anim. Vet. Sci*. 2 (10): 574-581. doi: | <http://dx.doi.org/10.14737/journal.aavs/2014/2.10.574.581>.
- Chang-Soo, K., Sung-Kwon, L., Tae-Oh, K., Sin-Ii, K. (1996). Détermination of sulfonamide residues in raw milk from southern Kyeonggi area. *Korean J. Vet. Serv*. 19: 39-45.
- Chris, M.L., Luke, C., Dietrich, A.V. (1999). Rapid analysis of tetracycline antibiotics by combined solid phase microextraction/ high performance liquid chromatography/mass spectrometry. *Rapid Communications in Mass Spectrometry*. 13(17): 1744-1754.
- Clifford, M.N. (1985). The History of Immunoassays in Food Analysis. In: *Immunoassays in Food Analysis*. Elsevier Applied Science Publishers, New York. 3-10.
- Conceição Luiz, D.A., Jos´ E Valenzuela Bell, L., Da Rocha, M., Nayara Lizandra, L., De Carvalho, D.A.V. (2018). Detection of veterinary antimicrobial residues in milk through near-infrared absorption spectroscopy. *Journal of Spectroscopy*. Vol 2018, Article ID 5152832, 6. <https://doi.org/10.1155/2018/5152832>.



- Dawadi, S., Thapa, R., Modi, B., Bhandari, S., Timilsina, A.P., Yadav, R.P., Aryal, B., Gautam, S., Sharma, P., Thapa, B.B. (2021). Technological advancements for the detection of antibiotics in food products. *Processes*. 9: 1500. <https://doi.org/10.3390/pr9091500>.
- Desalegne, A.S. (2011). Detection and determination of oxytetracycline and penicillin G antibiotic residue levels in bovine bulk milk from debrezeit and nazareth dairy farms. *Proceedings of the 1<sup>st</sup> International Technology, Education and Environment Conference (c) African Society for Scientific Research (ASSR)*.
- Dolliver H., Kumar, K., Gupta, S., Singh, A. (2008). Application of enzyme-linked immunosorbent assay analysis for determination of monensin in environmental samples. *Journal of Environmental Quality*. 37: 1220-1226.
- Elizabeta, D., Zehra, H., Biljana, S., Pavle, S., Risto, V. (2011). Screening of veterinary drug residues in milk from individual farms in Macedonia. *Mac. Vet. Rev.* 34(1): 5-13.
- Ergin, K.S. and Filazi, A. (2010). Determination of antibiotic residues in milk samples. *Kafkas Univ Vet Fak Derg.* 16(Suppl-A): 31-35.
- Fejzic, N., Begagic, M., Seric-Haracic, S., Smajlovic, M. (2014). Beta lactam antibiotics residues in cow's milk: Comparison of efficacy of three screening tests used in Bosnia and Herzegovina. *Bosn. J. Basic Med. Sci.* 14: 155-159.
- Ferraz, S.B., Marcus, A.G., Alves, A.J., Monteiro, M.A., Lima, A.M.B., Pereira, M.U., Luiz, R.A., Da Nóbrega, A.W. (2009). A liquid chromatography-tandem mass spectrometry confirmatory assay for the simultaneous determination of several tetracyclines in milk considering keto-enol tautomerism and epimerization phenomena. *Analytica Chimica Acta*. 656: 72-84.
- Franek, M. and Diblikove, I. (2006). Broad-specificity immunoassays for sulfonamide detection: Immunochemical strategy for generic antibodies and competitors. *Analytical Chemistry*. 78: 1559-1567.
- Furasawa, N. (2003). Isolation of tetracycline in milk using a solid-phase extraction column and watereluent. *Talanta*. 59: 155-159.
- Gardner, I.A., Cullor, J.S., Galey, F.D., Sischo, W., Salman, M., Slenning, B., Erb, H.N., Tyler, J.W. (1996). Alternatives for the validation of diagnostic assays used to detect antibiotic residues in milk. *J. Am. Vet. Med. Assoc.* 209: 46-52.
- Karamibonari, A.R. and Movassagh, H.M. (2011). Determination of tylosin residues by ELISA in pasteurized milk marketed in tabriz. *Global Veterinaria*. 6(6): 527-529.
- Khachatourians, G.G. (1998). Agricultural use of antibiotics and the evolution and transfer of antibiotic. *JAMC*. 159: 1128-1136.
- Khan, M.Z.H. (2020). Recent biosensors for detection of antibiotics in animal derived food. *Crit. Rev. Anal. Chem.* 1-11.
- Kümmerer, H.T.K. (2001). Pharmaceuticals in the environment-sources, fate, effects and risks. *Aquat Toxicol.* 71: 391-392.
- Lee, H.J., Lee, M., Han, K. (2000). Application of ELISA for the detection of penicillin antibiotic residues in live animal. *College of Agriculture and Life Sciences, Seoul National University. Suwon*. 441-744.
- Li, C., Zhang, Y., Eremin, S.A., Yakup, O., Yao, G., Zhang, X. (2017). Detection of kanamycin and gentamicin residues in animal-derived food using IgY antibody based ic-ELISA and FPIA. *Food Chem.* 227: 48-54.
- Moats, W.A. (2000). Determination of tetracycline antibiotics in beef and pork tissues using ion-paired liquid chromatography. *Journal of Agricultural and Food Chemistry*. 48(6): 2244-2248.
- Molina, M.P., Althausn, R.L., Molina, A., Fernández, N. (2003). Antimicrobial agent detection in ewes' milk by the microbial inhibitor test brilliant black reduction test-BRT AiM®. *Int. Dairy J.* 13: 821-826.
- Mukunzi, D., Isanga, J., Suryoprabowo, S., Liu, L., Kuang, H. (2017). Rapid and sensitive immunoassays for the detection of lomefloxacin and related drug residues in bovine milk samples. *Food Agric. Immunol.* 28: 599-611.
- Naoto, F. (1999). Rapid liquid chromatographic determination of oxytetracycline in milk. *Journal of Chromatography. A*(839): 247-251.
- Navratilova, P., Borkovcova, I., Drackova, M., Janstova, B., Vorlova, L. (2009). Occurrence of tetracycline chlortetracycline and oxytetracycline residues in raw cow's milk. *Czech J. Food Sci.* 27: 379-385.
- Pietschmann, J., Spiegel, H., Krause, H.J., Schillberg, S., Schroper, F. (2020). Sensitive aûatoxin B1 detection using nanoparticle-based competitive magnetic immunodetection. *Toxins*. 12(5): 337. <https://doi.org/10.3390/toxins12050337>.
- Popadoyannis, I.N., Samanidou, V.F., Kovatsi, L.A. (2000). A rapid high performance liquid chromatographic assay for the determination of oxytetracycline in commercial pharmaceuticals. *Journal of Pharmaceutical and Biomedical Analysis*. 23: 275-280.
- Ramasamy, T., Keerthana, S., Srinivasan, M.R., Chandrasekar, D., Porteen, K., Borthakur, A., Elamaran, A. and Sriram, P. (2021). Molecular Characterization of antibiotic resistance gene pattern of *Staphylococcus aureus* and *Escherichia coli* in mastitis affected dairy cows. *Indian Journal of Animal Research*. 55(4): 463-468. doi: 10.18805/ijar.B-3972.
- Romer, T., Van Weyenberg, S., Molina, M.P., Reybroeck, W. (2016). Detection of antibiotics in goats' milk: Comparison of different commercial microbial inhibitor tests developed for the testing of cows' milk. *Int. Dairy J.* 62: 39-42.
- Sabbya, S., Jannatul, F., Mahmudul, H.S., Azizul, K.H. (2019). Antibiotic residues in milk: Past, present and future. *Journal of Advanced Veterinary and Animal Research*. 6(3): 315-332. <http://doi.org/10.5455/javar.2019.f350>.
- Sarmah, A.K., Meyer, M.T., Boxall, A.B. (2006). A global perspective on the use, sales, exposure pathways, occurrence, fate and effects of veterinary antibiotics in the environment. *Chemosphere*. 65: 725-759.
- Shaheen, M., Tantary, H.A., Nabi, S.A. (2016). Treatise on bovine mastitis: Disease and disease economics, etiological basis, risk factors, impact on human health, therapeutic management, prevention and control strategy. *J. Adv. Dairy Res.* 4(1): 1-10.
- Shen, X., Liu, L., Xu, L., Maw, L., Wu, X., Cui, G., Kuang, H. (2019). Rapid detection of pra ziquantel using monoclonal antibody-based ic-ELISA and immunochromatographic strips. *Food Agric. Immunol.* 30: 913-923.

- Simonsen G.S., Haaheim, H., Dahl, K.H., Kruse, H., Lovseth, A., Olsvik, O., Sundsfjord, A. (1998). Transmission of Van samples A-type vancomycin resistant enterococci and Van A resistance elements between chicken and humans at avoparcin-exposed farms. *Microb Drug Resist* Winter. 4 (4): 313-318.
- Szekacs, A. (1994). Development of enzyme-linked immunosorbent assay (ELISA) systems for environmental monitoring. *Acta Biol. Hung.* 45: 77-80.
- Unusan, N. (2009). Occurrence of chloramphenicol, streptomycin and tetracycline residues in ultra-heat treatment milk marketed in Turkey. *Int J. Food Sci Nutr.* 60(5): 359-64.
- Valarmathi, P. (2020). Antibiotics-miracle drugs as crop protectants: A review. *Agricultural Reviews.* 41(1): 43-50. doi: 10.18805/ag.R-1941.
- Van Boeckela, T., Brower, C., Gilbert, M., Grenfell, B.T., Levin, S.A., Robinson, T.P., Teillant, A., Laxminarayan, R. (2015). Global trends in antimicrobial use in food animals. *PANS.* 112(18): 5649-5654.
- Vatalia, D.J., Bhanderi, B.B., Nimavat, V.R. and Jhala, M.K. (2021). Isolation, molecular identification and multidrug resistance profiling of bacteria causing clinical mastitis in cows. *Agricultural Science Digest.* 41(2): 345-351. doi: 10.18805/ag.D-5220.
- Waghamare, R.N., Paturkar, A.M., Vaidya, V.M., Zende, R.J., Kumar, A. and Bedi, J.S. (2020). Screening of antimicrobial residues and confirmation of doxycycline in samples collected from chicken farms and processing units located around Mumbai, India. *Indian Journal of Animal Research.* 54(11): 1415-1421. doi: 10.18805/ijar.B-3899.
- Zahid, H.S.M., Mostafa, K.A.T.M., Sabuj, B.M., Sheikh, A.M.D., Kishor, M., Hassan, K.M.D., Arifuzzaman, M.D. (2010). Detection of residual antibiotics in fresh cow milk. *Bangladesh Pharmaceutical Journal.* 13(2): 64-66.
- Zhang, H. and Wang, S. (2009). Review on enzyme-linked immunosorbent assays for sulfonamide residues in edible animal products. *Journal of Immunological Methods.* 350: 1-13.
- Zhang, Y.D., Zheng, N., Han, R.W., Zheng, B.Q., Yu, Z.N., Li, S.L., Zheng, S.S., Wang, J.Q. (2014). Occurrence of tetracyclines, sulfonamides, sulfamethazine and quinolones in pasteurized milk and UHT milk in China's market. *Food Control.* 36(1): 238-242.